Funcionalidad celular

El citómetro de flujo tiene la capacidad única de medir parámetros fisiológicos de gran número de células individuales

Las células se estudian en su “estado de base” y luego de la introducción del estímulo

Viabilidad celular

Table 9.1.1 Probes for Cell Viability

Probe	Excitation wavelength (nm)	Emission wavelength (nm)
Dye-exclusion probes		
Propidium iodide	488	>590
7-amino actimomycin D	488	>650
Functional probes		
Fluorescein diacetate	488	520-540
Rhodamine 123	488	520-540
Probes usable with fixed cells		
Ethidium monoazide	488	>630
LDS-751	488	>650

ELIMINACION DE CELULAS MUERTAS DE UNA SUSPENSION CELULAR

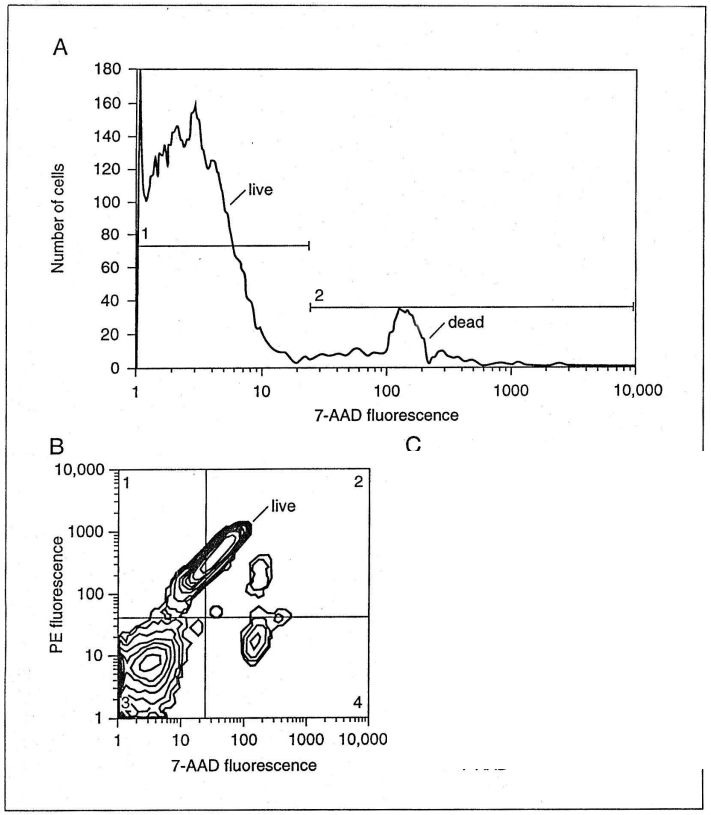
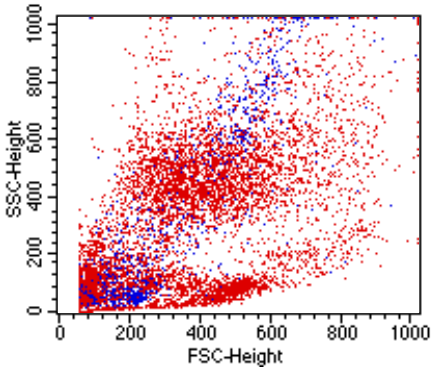
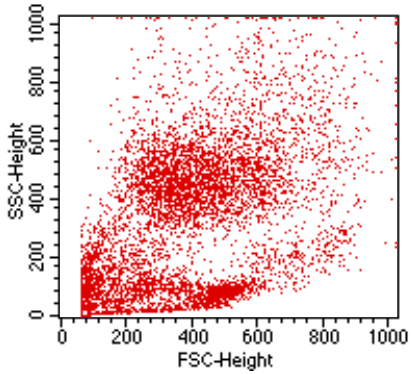
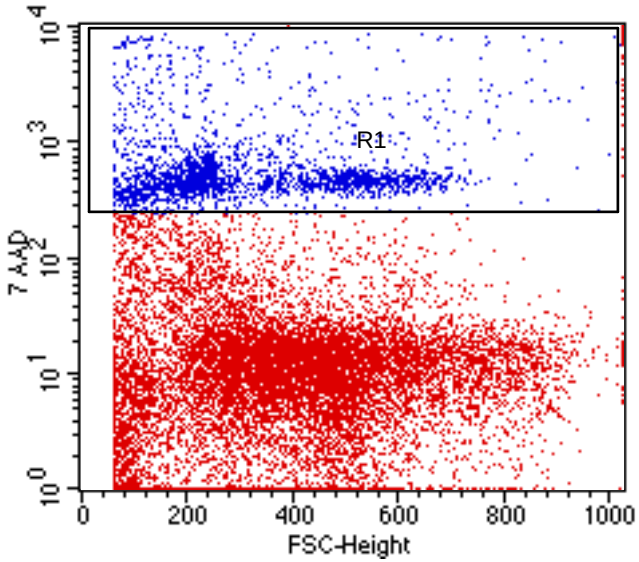


Figure 9.2.2 Effects of gating and compensation with 7-AAD. (**A**) Gating discriminates live cells. One-parameter histogram of logarithmically amplified 7-AAD fluorescence using a 650-nm long-pass filter. Mouse spleen cells are labeled only with 7-AAD. Note the peak of dead cell population in region 2 at a relative brightness between 100 and 200 (about ten-fold dimmer than what is expected for propidium iodide). The live cell population that occupies the first decade in the histogram (region 1) is 7-AAD negative and constitutes the majority of the cells in the population. (**B**) Uncompensated phycoerythrin fluorescence in the presence of 7-AAD. A bivariate plot of mouse spleen cells labeled with 7-AAD and a PE-labeled antibody to a cell surface antigen. Note the two small populations of dead cells in regions 2 and 4 and the large population of live cells that occupies region 2. If gating was performed before adequate compensation was achieved, then most of the viable PE-positive cells could be lost. (**C**) Compensation of PE with 7-AAD. Distribution of cells from same sample as in B. Note the dead cells are in the same location as in B, but the live cells are now clearly resolved from 7-AAD positive populations. At this point, the live cell gate defined in region 2 of A is valid.







Ca^{2+} intracitoplasmático

Table 9.1.4 Calcium Indicator Dyes Useful for Flow Cytometric Applications

Indicator	Emission response to elevated calcium	Excitation wavelength (nm)	Emission wavelength (nm)	Calcium affinity, K_d (nM)[a]	
				22°C	37°C
Indo-1	Ratio	325-360	390/520	NA	~250
Fluo-3	Increase	488	530	~400	~860
Calcium Green-1	Increase	488	530	~250	NA
Calcium Orange	Increase	550	575	~330	NA
Calcium Crimson	Increase	590	610	~200	NA
Fura Red	Decrease	488	660	~400	NA
Fluo-3/Fura Red	Ratio	488	530/660	~400	NA

GRUPO RIOPLATENSE DE CITOMETRIA DE FLUJO
IMEX CONICET A N M
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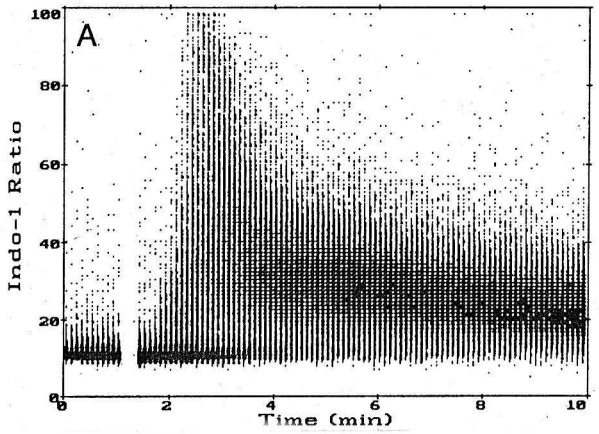
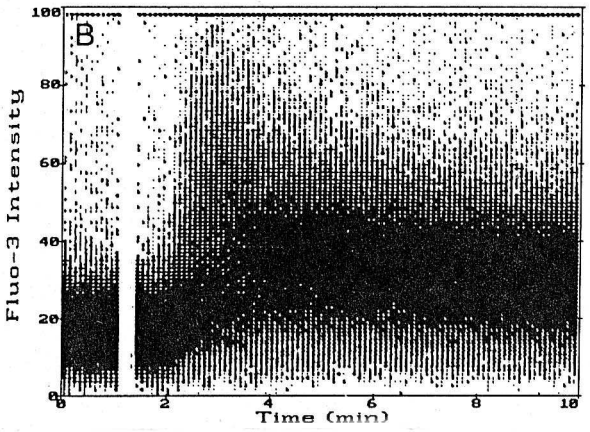
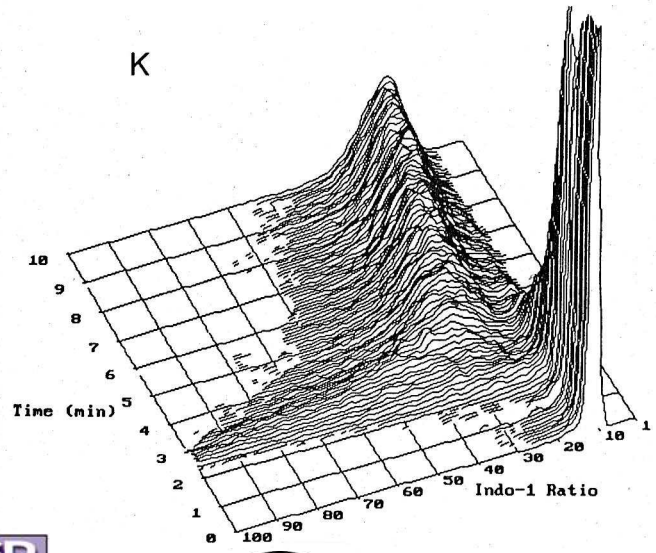
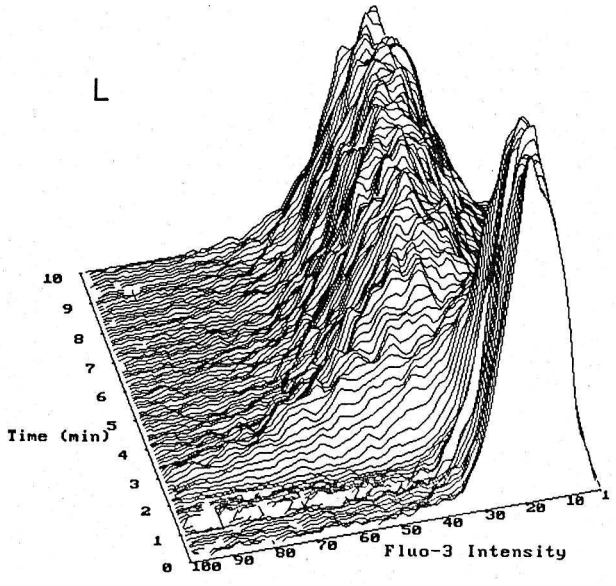
ANALISIS DEL [Ca2+] INTRACELULAR









GRUPO RIOPLATENSE DE CITOMETRIA DE FLUJO

IMEX CONICET A N M

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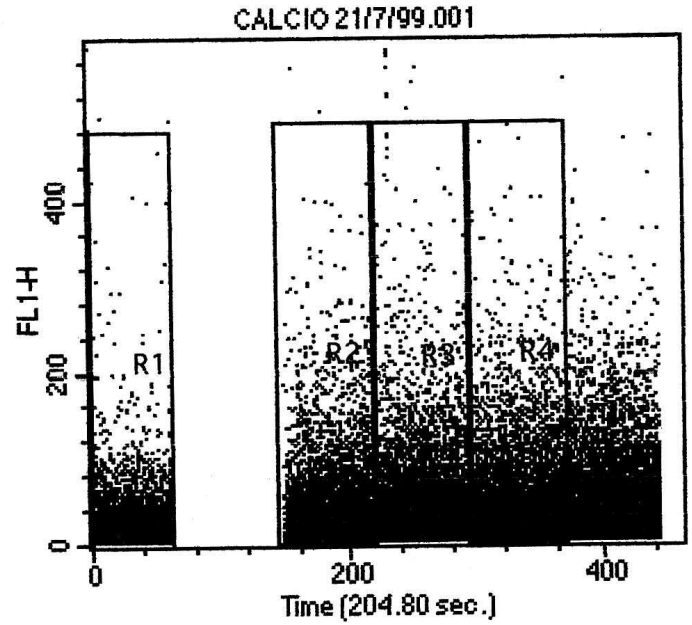
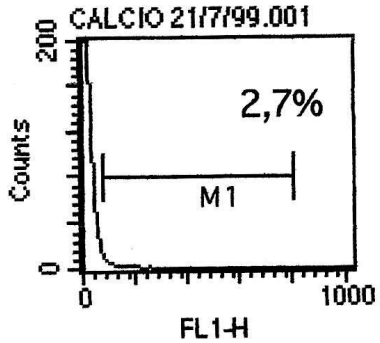
Análisis de los datos

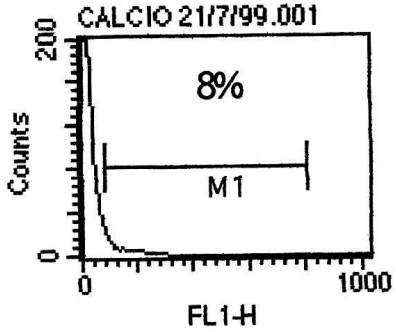


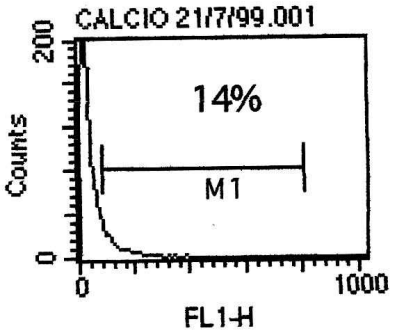
Se puede agregar probenecid en caso de células que no se colorean bien ya que evita la difusión del colorante hacia el medio externo.

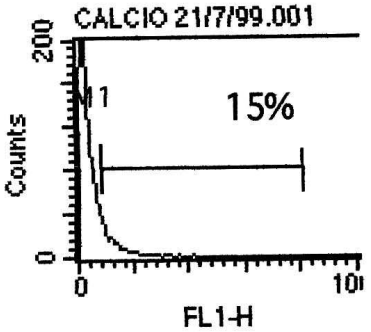
Es cualitativo:

se compara:
basal con estimulado.
tratado o sin tratamiento









Metabolismo oxidativo

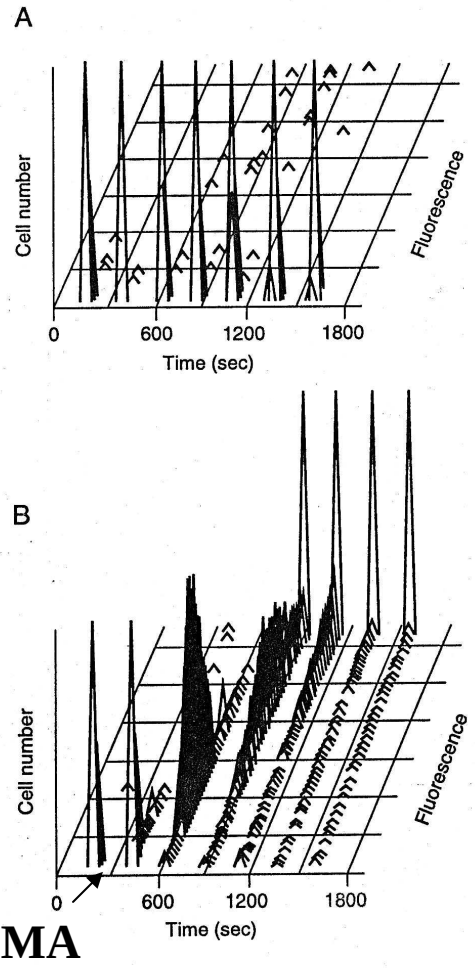
Algunas células producen gran cantidad de radicales del oxígeno debido a su rol protector del ataque de microorganismos.
La medición intracelular de la producción de estas moléculas nos da información sobre la habilidad de las células para operar.
Se emplea en pacientes con diagnóstico clínico de disfunción fagocítica, en el diagnóstico de enfermedad granulomatosa crónica.

Table 9.1.2 Probes for Oxidative Metabolism

Probe	Excitation wavelength (nm)	Emission wavelength (nm)
Dyes for H_2O_2		
Dichlorofluorescin diacetate	488	525
Dihydrorhodamine 123	488	525
Dyes for O_2^-		
Hydroethidine	488	590

La generación de IROs oxida las sondas no fluorescentes a especies fluorescentes

PRODUCCION DE PEROXIDO DE HIDROGENO VALORADA CON DCFH-DA

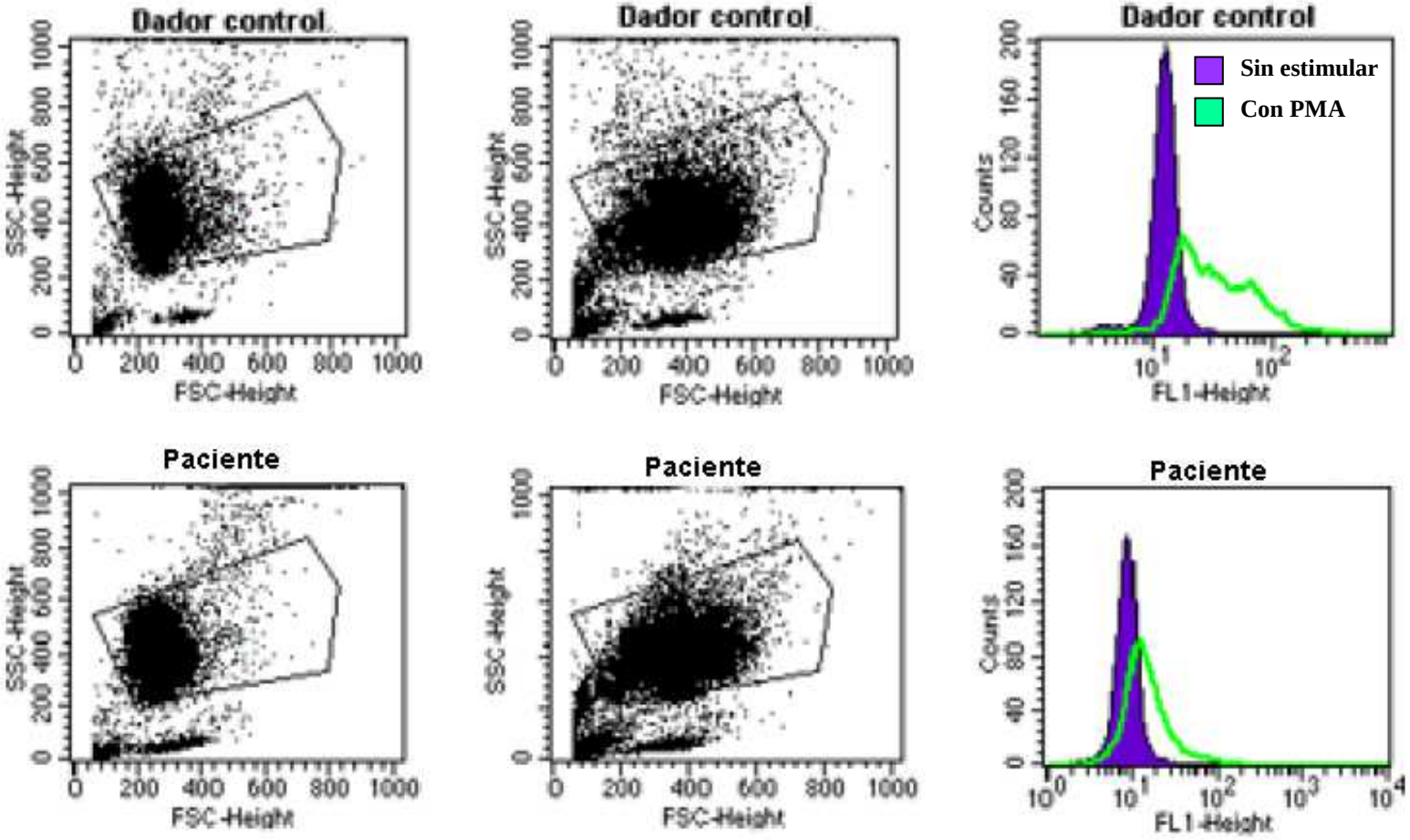


Polimorfonucleares sin estimular

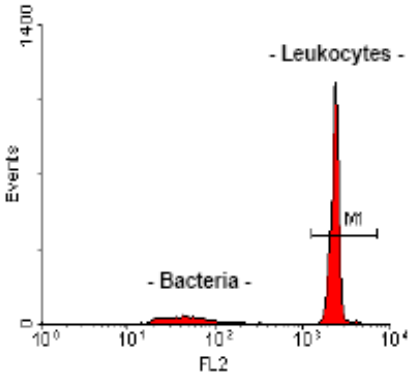
Polimorfonucleares estimulados con PMA

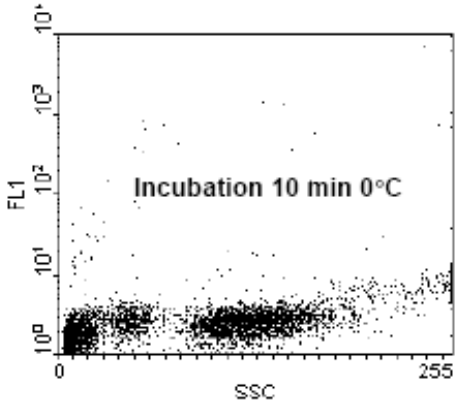
Figure 9.7.3 Stimulation of neutrophils by PMA over time. (**A**) Unstimulated cells, which show no increase in DCF fluorescence over 30 min. (**B**) Activated cells (stimulated with 50 ng/ml PMA), which show a rapid 10-fold increase in fluorescence following an initial time lag. Data were collected by sampling the same set of tubes multiple times over a 30-min period.

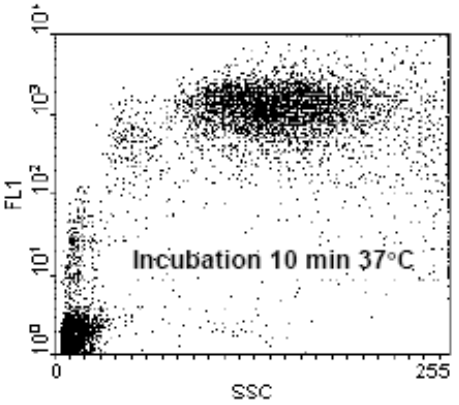
123-Dihydrorhodamina en la evaluación de estallido respiratorio en granulocitos

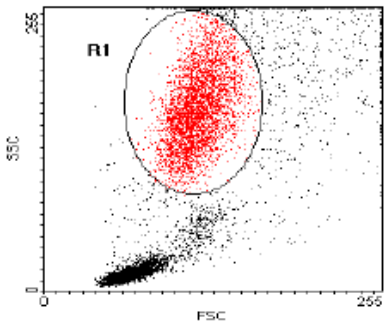


Fagocitosis-Endocitosis

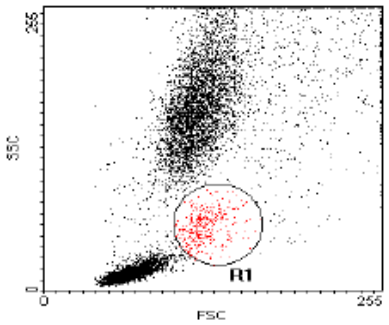


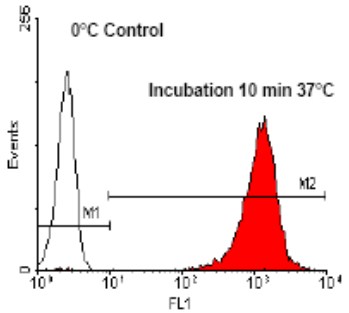


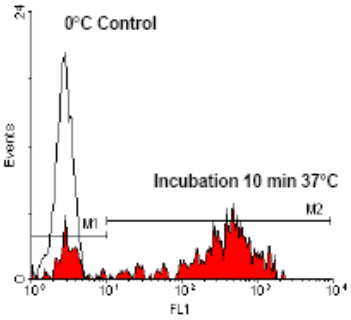




B) Gate set on **monocytes**







SE (hep) + partículas FITC(opsoniz. o no)

10 minutos | 0°C
37°C

↓

Solución de Quenching
(azul tripan o cristal violeta)

↓

Lavar 2 veces

↓

Lisar los GR y fijar

↓

Lavar 2 veces

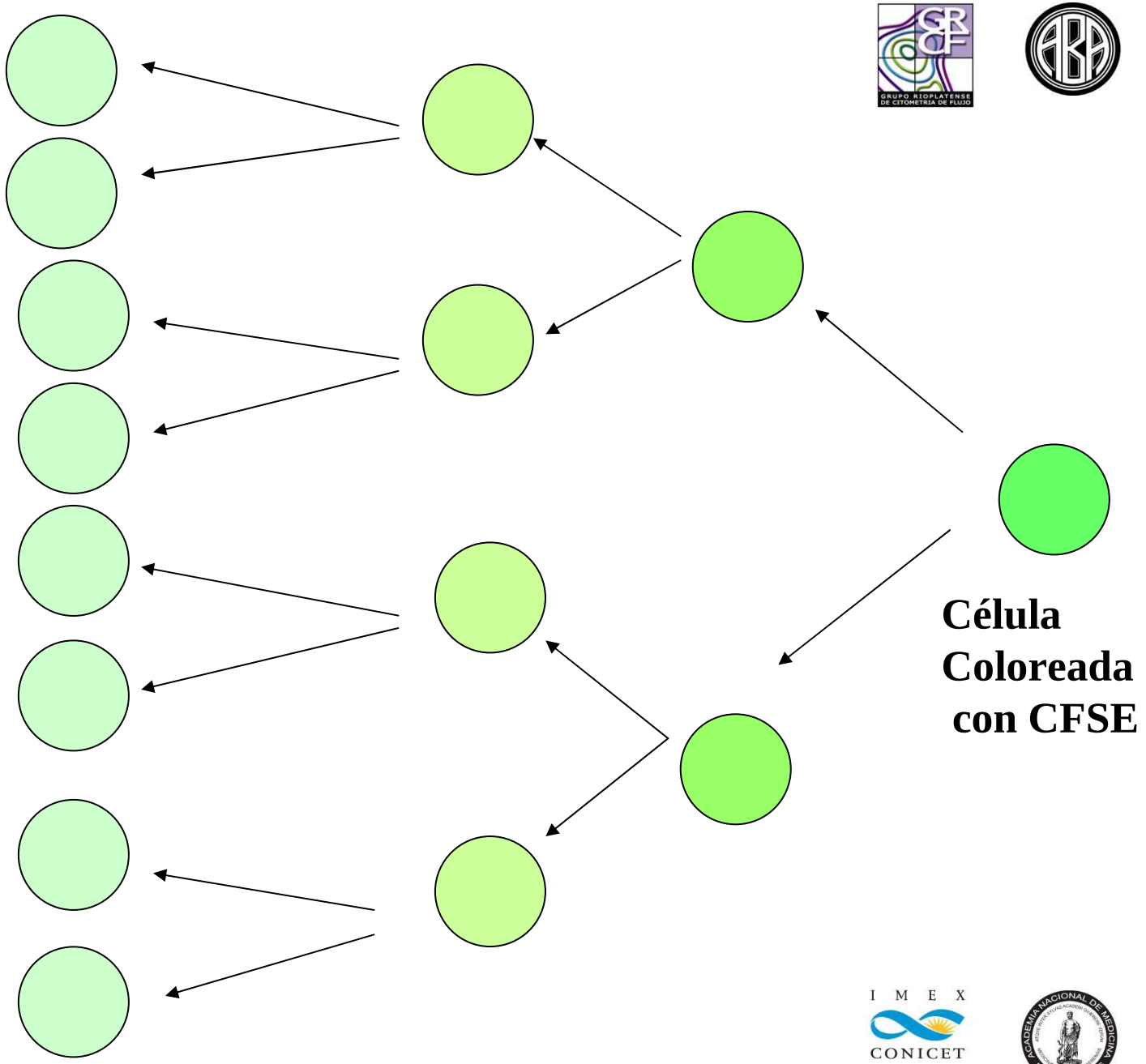
↓

Teñir DNA

Estudio de la proliferación celular en respuesta a un estímulo

Util para detectar células específicas (LT o LB) respondedoras a un estímulo

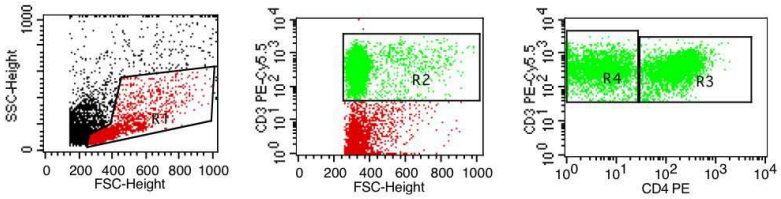
Evita el uso de sustancias radioactivas.



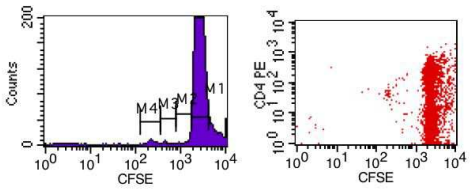
CFSE= 5,6- carboxyfluorescein diacetate succinimidyl ester

Medida de la proliferación celular en respuesta a un estímulo utilizando el colorante CFSE

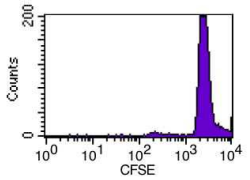
Linfocitos estimulados con PHA. Marcación con CFSE/anti-CD4 PE/anti-CD3 PE Cy5

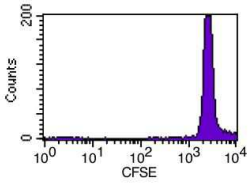


Sin estimular

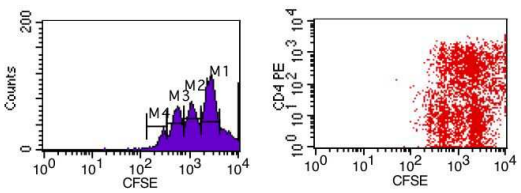


Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	28514	100.00	53.30	2509.58
M1	1670, 3995	26446	92.75	49.43	2365.38
M2	764, 1670	505	1.77	0.94	1530.41
M3	346, 764	60	0.21	0.11	507.29
M4	129, 346	156	0.55	0.29	216.64



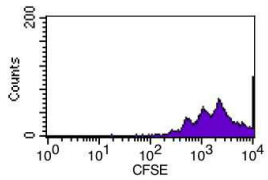


con PHA

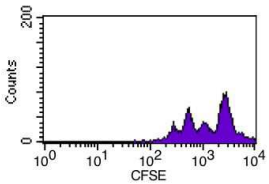


R1 AND R2: Total de CD3+

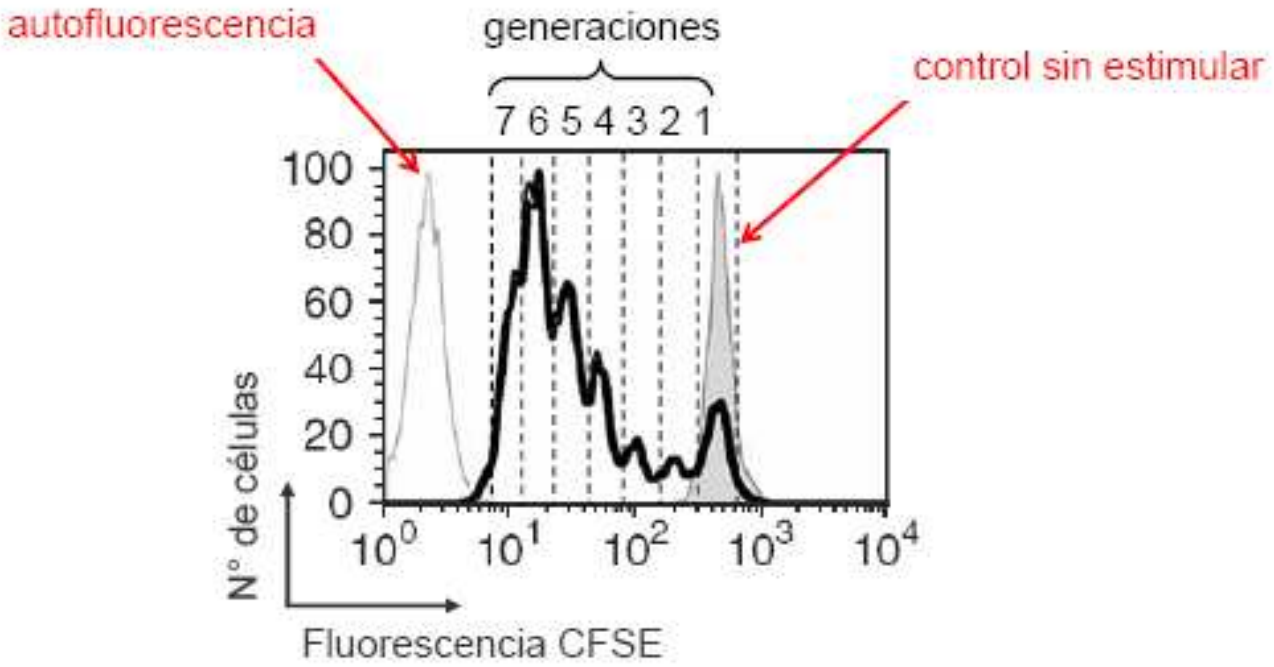
Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	16482	100.00	30.56	2098.85
M1	1670, 3995	6592	40.00	12.22	2514.24
M2	764, 1670	3918	23.77	7.27	1137.44
M3	346, 764	3257	19.76	6.04	528.41
M4	129, 346	949	5.76	1.76	264.40



R1 AND R3: CD4+



R1 AND R4: CD4-



En un estudio multiparamétrico se pueden estudiar las características de cada generación

Fin Tercera Parte

Muchas gracias